

INTRACELLULAR DIGESTION OF SYMBIONTIC ZOOXANTHELLAE
BY HOST AMOEOCYTES IN GIANT CLAMS (BIVALVIA:
TRIDACNIDAE), WITH A NOTE ON THE NUTRI-
TIONAL ROLE OF THE HYPERTROPHIED
SIPHONAL EPIDERMIS

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Zooxanthellae are highly specialized dinoflagellates which live mostly as endosymbionts within a wide phyletic range of marine invertebrate hosts (Buchner, 1965; McLaughlin and Zahl, 1966). The symbiosis between zooxanthellae and host animal is regarded by most workers as mutualistic; that is, a relationship which is beneficial to both host and symbiont. In exchange for protection, carbon dioxide, and nutrient salts provided by the host tissues, the symbiont releases small amounts of oxygen and an appreciable quantity of photosynthetic metabolites (principally glycerol) which are utilized by the host (Smith, Muscatine and Lewis, 1969; Goreau, Goreau and Yonge, 1965; Yonge, 1944).

Within the family Tridacnidae (see Fig. 1 for an example of this group), this relationship is further elaborated. From histological evidence, Yonge (1936 and 1953) has concluded that zooxanthellae are conveyed within amoebocytes from the hypertrophied siphonal haemal spaces of *Tridacna* where they are "farmed" by the clam, to the interdiverticular spaces of the clam's digestive gland. Further, these engulfed zooxanthellae are intracellularly digested by the amoebocytes both *en route* via blood vessels from the mantle and within the interdiverticular spaces of the digestive gland.

Mansour's accounts (1945, 1946 and 1949) are in disagreement with Yonge's findings. Zooxanthellae were never found within the amoebocytes of tridacnid clams by Mansour. Moreover, it was solely in young or juvenile tridacnids that zooxanthellae were observed by him within tissues of the visceral mass. In no case were algal cells present in blood vessels; *ergo*, concludes Mansour, zooxanthellae could not, as claimed by Yonge (1936 and 1953), be transported from the siphons to the visceral mass via the circulatory system.

The utilization of symbiotic algae as a holozoic food source by their invertebrate hosts has been recently reviewed (Smith, Muscatine and Lewis, 1969), and with regard to the acquisition of insoluble carbohydrate by the host as a result of degeneration and digestion of whole algal cells or their parts, it was stated that the critical evidence is still lacking. The purpose of this report is, hopefully, to settle this question, at least with respect to tridacnid clams, by means of the more precise techniques of electron microscopy and electron microscopical histochemistry. In addition, the possible nutritive role of the epidermis covering the hypertrophied siphons will be discussed.

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FIGURE 1. In water photograph of one of the more diminutive species of tridacnid clams, *Tridacna maxima* taken adjacent to Chinimi Islet, Eniwetok Atoll, Marshall Islands. The clam lies byssally attached to coral incrusting limestone with its hypertrophied siphons (the whole of the fleshy portion in the figure) fully exposed to the sun's rays.

MATERIALS AND METHODS

Adult specimens of the tridacnid clams *Hippopus hippopus*, *Tridacna gigas*, *T. maxima*, and *T. squamosa* (Rosewater, 1965), were collected in shallow coral reef waters at Eniwetok Atoll, Marshall Islands, by free diving or using S. C. U. B. A. Blood samples from the circulatory systems were syringe extracted from the clams' hearts and immediately centrifuged to precipitate a firm easily handled pellet of amoebocytes. One millimeter square cubes of clam digestive gland and the mantle edge inner folds (*Hippopus* excluded) were cut from tridacnids and, along with the centrifuged amoebocyte fractions, were treated in one or both of the following ways:

Method I—light and electron microscopy

1. Fixed at 0° C for 1½ hours in 2% osmium tetroxide buffered with Dorey's solution B (1965). For better penetration and osmotic compatibility of fixative with tissues, 3.5% potassium chloride was substituted for the sucrose originally prescribed in the Dorey formula.

2. Dehydrated and embedded with Epon 812 (Luft, 1961).

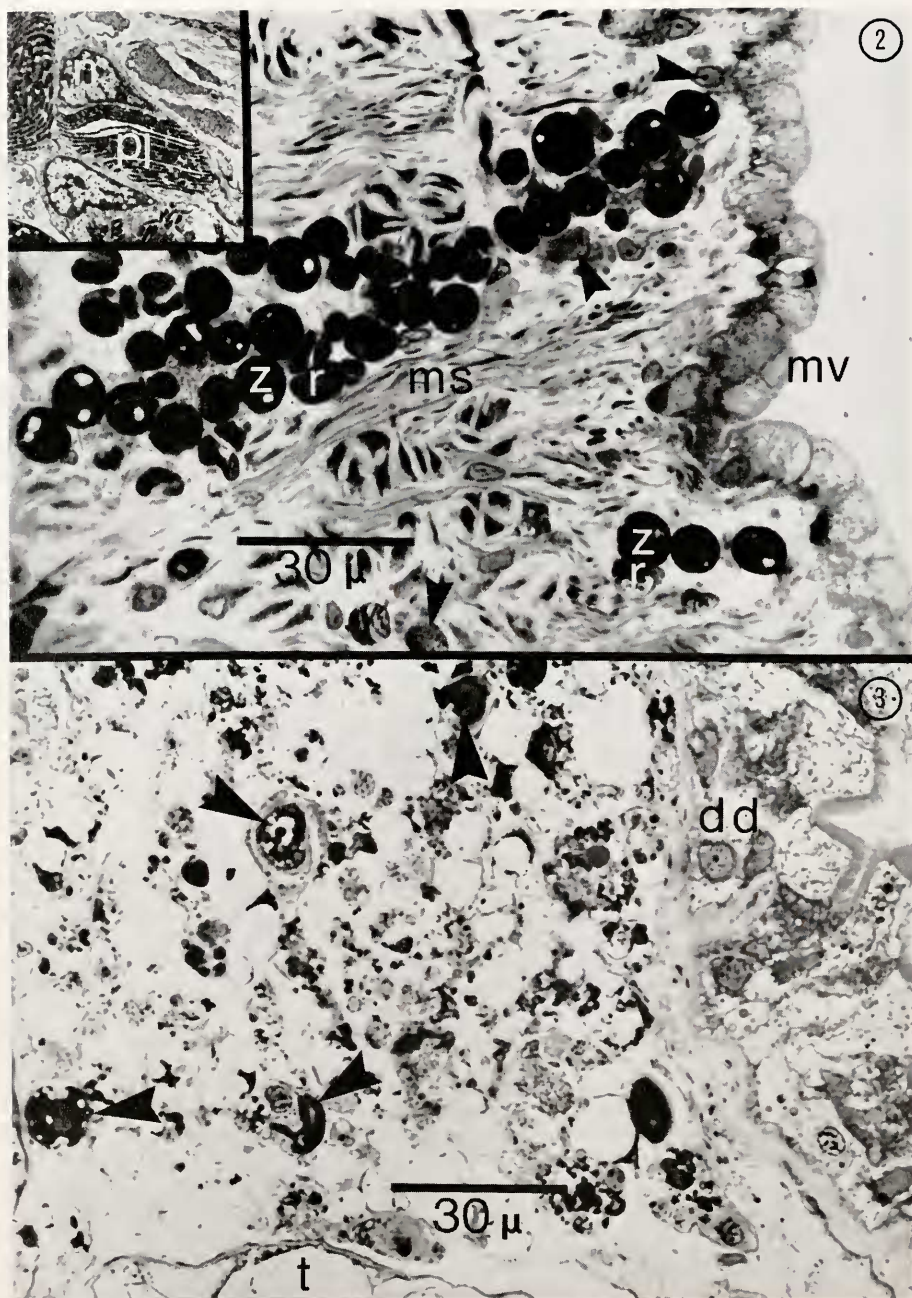


FIGURE 2. Siphonal tissue of *Tridacna maxima* hosting symbiotic zooxanthellae within baemal channels. Insert top left: Fine structure of iridophore from siphonal tissues. Abbreviations used are: ms, muscle strands; mv, microvillous surface of vacuolated epidermal cells; n, nucleus; pl, plates; r, iridophore; z, zooxanthellae; point, amoebocyte.

3. Gray to silver sections were cut and stained with lead and uranium for electron microscopy. For conventional bright field light microscopy, one micron thick sections were cut, affixed to microscope slides and stained with Richardson's stain (Richardson, Jarett, and Finke, 1960).

Method II—histochemical test for phosphomonoesterase II (acid phosphatase)

1. Fixed at 0° C for 2 hours in 6% glutaraldehyde buffered with 0.2 M cacodylate at pH 7.4. The 6% glutaraldehyde solution was made from a 25% prebuffered (3% calcium carbonate) stock solution of "Fisher" biological grade glutaraldehyde.

2. Washed at 0° C overnight in 0.2 M cacodylate buffer (pH 7.4.).

3. Incubated in fresh filtered Gomori's medium (1950) at 37° C for 1½ hours. The β -glycerophosphate constituent of the Gomori medium should contain no more than 0.1% L- α -isomer impurity for best results.

4. Washed as follows: acetate buffer, pH 5.0, 15 minutes; 2% acetic acid, 3 minutes; acetic acid, 3 minutes; acetate buffer, pH 7.2, 10 minutes.

5. Postosmicated, dehydrated, and embedded as described in Method I, above.

6. For electron microscopy, silver-gold thin sections were cut and left unstained.

7. The control incubation medium consisted of the Gomori medium with 0.42% sodium fluoride added as an enzyme inhibitor.

I have drawn liberally upon the papers of Kevin, Hall, McLaughlin and Zahl (1969) and Taylor (1969a) for identification of fine structure in zooxanthellae.

RESULTS

The hypertrophied siphonal tissues of *Tridacna* are comprised chiefly of a muscle and connective tissue meshwork covered by a narrow undulant veneer of highly vacuolated microvillous epidermal cells (Fig. 2). Symbiotic zooxanthellae live closely appressed within host haemal channels, which are arranged approximately perpendicular to the siphon's exposed surface. Scattered amongst the zooxanthellae (z) are various amoebocytes (a) and iridophores (r). Peculiar to the cytoplasm of iridophores are stacks of parallel electron dense plates (pl) (Fig. 2) to which Kawaguti (1966) attributes the siphon's iridescence.

Light microscope studies revealed that zooxanthellae of the interdiverticular spaces of the digestive gland (Fig. 3) and circulatory system are dissimilar in their gross histology from those found in the haemal channels of the siphon's. Algal cells from the siphons are uniformly ovoid in shape and possess a homogeneous cytoplasm rift with chloroplasts. In contrast to this, zooxanthellae seen in the circulatory system and seen in the interdiverticular spaces of the digestive gland may be observed in various conditions: ranging from free, inclusion-bearing orbs to barely recognizable deformed bodies which are undergoing the later stages of intracellular digestion by amoebocytes.

Ultrastructure of zooxanthellae living within tridacnid siphonal tissues

Electron photomicrographs demonstrated that siphonal zooxanthellae are adjacent to but never completely enclosed by amoebocytes (a), muscle strands (m),

FIGURE 3. Amoebocyte packed interdiverticular space of digestive gland of *Tridacna squamosa*. Abbreviations used are: dd, duct of digestive diverticula; t, tubule of digestive diverticula; points, digesting zooxanthellae contained within amoebocyte digestive vacuoles.

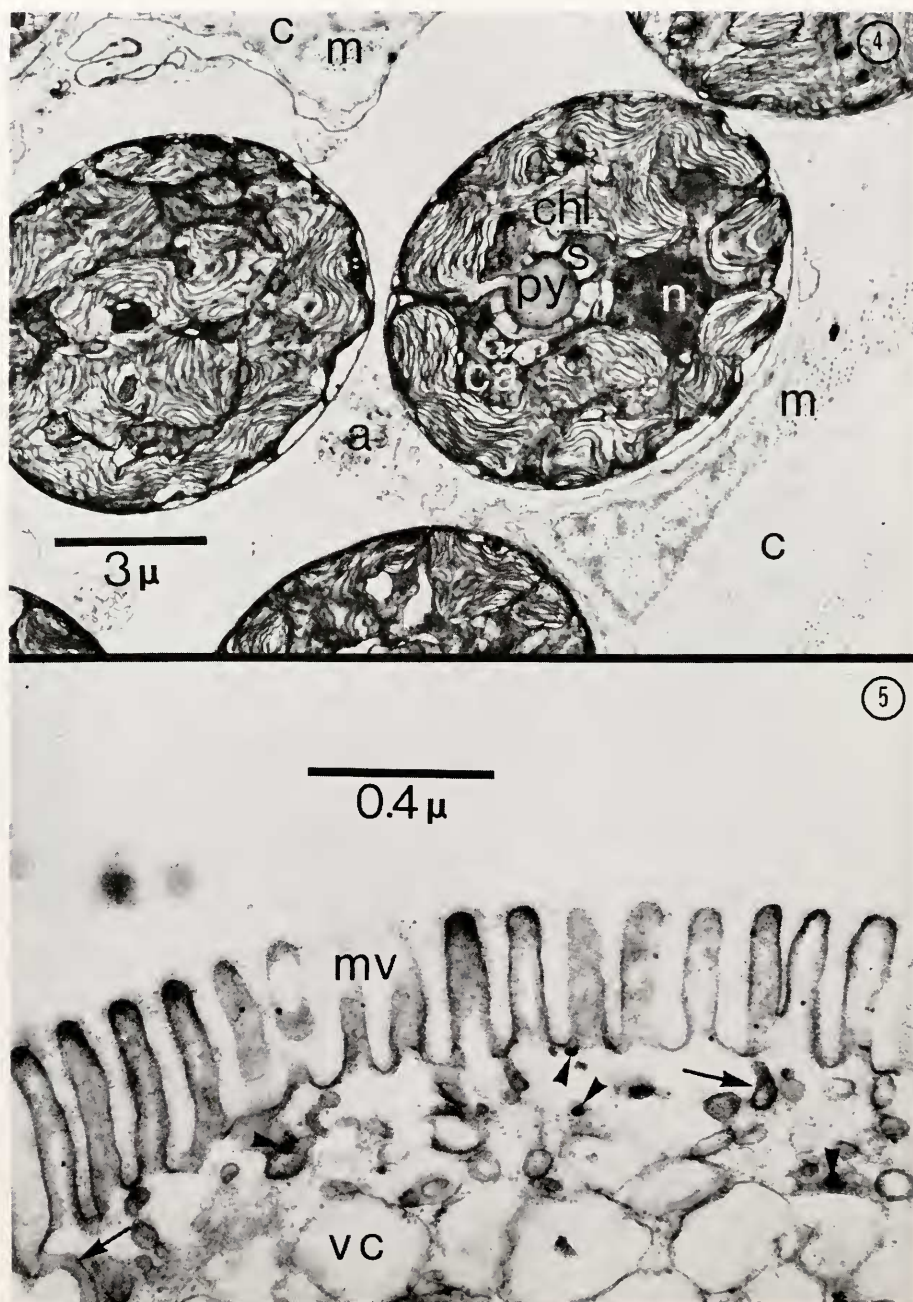


FIGURE 4. Ultrastructure of zooxanthellae within siphonal haemal space of *Tridacna maxima*. Note that zooxanthellae are adjacent, but not engulfed by amoebocytes. Abbreviations used are: a, portion of amoebocyte; c, connective tissue; ca, calcium oxalate crystals; chl, chloroplast; m, muscle strand; n, nucleus; py, pyrenoid body; s, starch cap.

connective tissue (c) (Fig. 4), or iridophores. Three unit membranes, comprising the zooxanthella's periplast, surround the chloroplast-dominated cytoplasm of the alga. The chloroplasts (chl) are individually joined by a short neck to a starch-capped pyrenoid body (py). Other cytoplasmic organelles which may be included are: a stellate-shaped nucleus (n), invested with numerous spring-shaped chromosomes; a small waste vacuole containing calcium oxalate crystals (ca); mitochondria, frequently multilobed; a small electron dense accumulation body; and, often, a four to six dictyosome golgi complex. I observed no obviously degenerate or senile zooxanthellae within siphonal tissues.

Uptake by microvilli covering the epidermis of tridacnid siphons

Microvillous epidermal cells of the hypertrophied siphons of *Tridacna* pinocytose phenomenal quantities of both fluid and particulate substances from the seawater which bathes the siphon's surface (Fig. 5). I use the term phenomenal advisedly because by my rough but conservative estimates, a 20 centimeter long specimen of *Tridacna maxima* possesses a total exposed siphonal surface area of over 1000 square centimeters. I could see evidence of fusion of pinocytotic vesicles with the epidermal cell's omnipresent clear cytoplasmic vacuoles, but the ultimate disposition of this endocysted material was not determined.

Intracellular digestion of zooxanthellae by amoebocytes lying within the interdiverticular spaces of the tridacnid digestive gland

The interdiverticular spaces of the tridacnid digestive gland are solidly packed with amoebocytes, many of which contain one or two zooxanthellae in various stages of intracellular digestion (Yonge, 1936). Free, or non-digesting, zooxanthellae are not commonplace in either the digestive gland or the circulatory system, but they do infrequently occur. However, whether free or engulfed, provided that, in the latter instance, intracellular digestion has not progressed beyond the early stages, nearly all of these zooxanthellae share recognizable cytological characteristics not common to their counterparts in the mantle edge. For example, the outer covering, or periplast, is greatly thickened due to the addition of a wide band of semi-opaque material sandwiched between the three original bounding membranes plus one or two more coated onto the periphery (Fig. 6). Another obvious periplast feature of many of the zooxanthellae undergoing digestion is the presence of vacuole-like foldings on the inside boundary of the opaque layer (Fig. 8). Presumably these structures are derivatives of the original periplast membranes. The chloroplasts (chl), now more electron dense than those seen in the zooxanthellae of the mantle, no longer predominate in the cytoplasm, but have been, in part, supplanted by much enlarged accumulation (ab) and calcium oxalate (ca) crystal bodies (Figs. 6 and 7). The pyrenoid body is still in evidence in some sections, but the starch cap has been assimilated by the zooxanthella. In many cases, lipid storage bodies are found throughout the cytoplasm.

Intracellular digestion of a zooxanthella by an amoebocyte is evidently initiated

FIGURE 5. Pinocytosis of fluid and particulate matter by siphonal microvillous epidermal cells in *Tridacna maxima*. Abbreviations used are: mv, microvillous surface of vacuolated epidermal cells; vc, clear vacuoles; arrows, fluid filled pinocytotic channels; points, pinocytosis of particulate material.

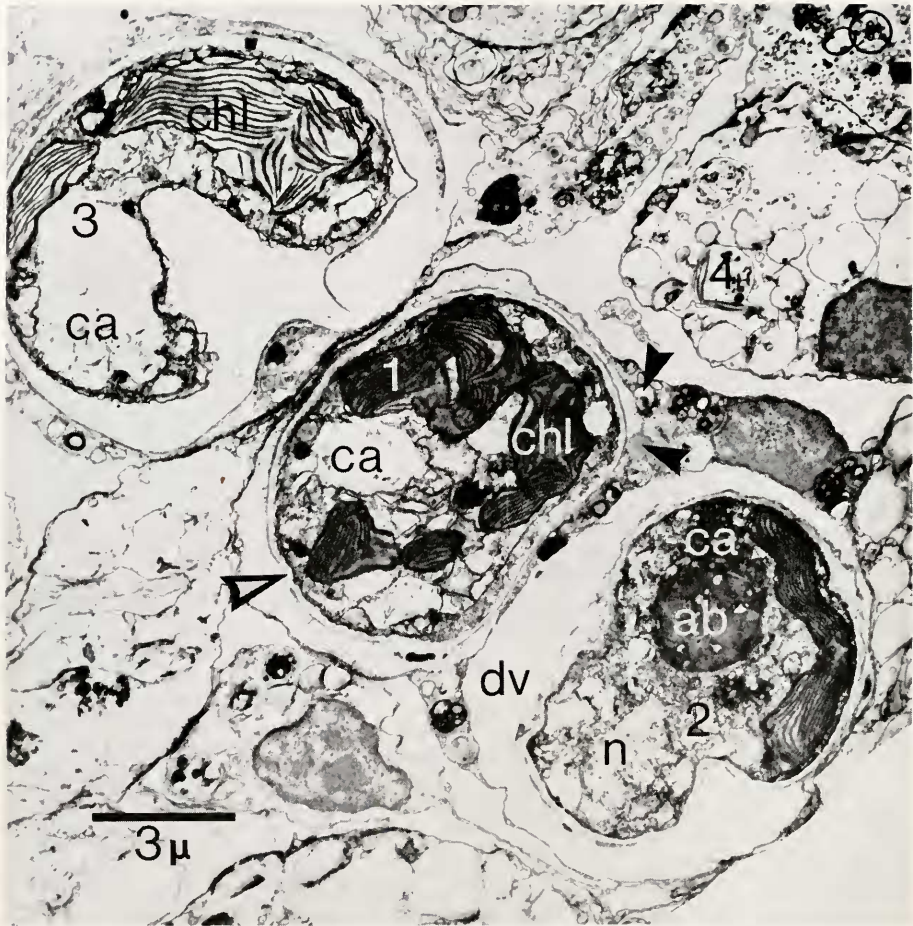


FIGURE 6. Enumerated successive stages of intracellular digestion of senescent zooxanthellae by amoebocytes lying within the interdiverticular spaces of the digestive gland of *Tridacna gigas*. (1), amoebocyte lysosomes burst their contents into vacuole containing the algal cell; (2), zooxanthella's periplast breaks down and cell outline becomes noticeably crenated; (3), chloroplast lamellae spread apart giving an overall bleached appearance to the zooxanthella; (4), digestive vacuole becomes reduced in size due to seepage of fluid nutrients to the amoebocyte's cytoplasm. Abbreviations used are: ab, accumulation body; ca, calcium oxalate crystals; chl, chloroplast; dv, amoebocyte digestive vacuole; n, nucleus; black points, amoebocyte lysosomes bursting contents into digestive vacuole; white on black point, amorphous layer of thick periplast.

when the amoebocyte's lysosomes burst their contents into the vacuole containing the algal cell; this vacuole is now termed the digestive vacuole (Fig. 6). Lysosome involvement in the digestive process was verified according to the terms of de Duve (1963) on the basis of both morphology and a positive histochemical test for the presence of phosphomonoesterase II (Fig. 7). In the second stage of intracellular digestion, the zooxanthella's periplast begins to break down and the algal cell has become noticeably crenated. Chloroplasts remain quite electron dense,

but the general cytoplasmic detail has become muddled. The membranes enclosing the calcium oxalate crystal bodies have begun to part, appearing to release the vacuole contents into the cytoplasm (Figs. 6 and 8). An abrupt alteration takes place in the electron density of and in the degree of apposition of the chloroplast lamellae in the third stage of the digesting zooxanthella. Chloroplast lamellae now possess a bleached appearance and are widely separated. As digestion progresses, the amoebocyte's digestive vacuole(s) (dv) contents become much reduced in size due, presumably, to the seepage of the fluid nutrient matter from the digestive vacuole to the cytoplasm of the amoebocyte. The final visible remains are barely recognizable bits of chloroplast thylakoids, and these are soon reduced to myelin figures (d) (Fig. 8) in the now diminutive digestive vacuole.

Light and electron microscope observations on sections cut of the amoebocyte fraction of centrifuged clam blood revealed numerous zooxanthellae, many of which were in early stages of digestion within amoebocyte digestive vacuoles. However, the relative numbers of zooxanthella to total blood cells from the circulatory system was noticeably more sparse than that found within the interdiverticular spaces of the digestive gland.

Reproduction of zooxanthellae (binary fission) was frequently observed in mantle edge tissue preparations, but in no instance did I find any suggestion of algal reproduction in tissues from either the digestive gland or the circulatory system.

DISCUSSION

Senescence in a vegetative zooxanthella may be recognized by the following characteristics: a low chloroplast density; large accumulation and calcium oxalate crystal bodies; a thick periplast formed by five unit membranes; and a pyrenoid body (if present) with a reduced or missing starch cap (Freudenthal, 1962; Kevin, Hall, McLaughlin and Zahl, 1969; Taylor, 1968). These criteria coincide with my description and/or electron photomicrographs (Figs. 3, 6, 7 and 8) of zooxanthellae observed from both the circulatory system and the interdiverticular spaces of the digestive gland of tridacnid clams. Hence, it is apparent that amoebocytes in tridacnids phagocytose mostly old or degenerate zooxanthellae from the algal population of the mantle edge.

Yonge (1936) has suggested that tridacnid clams "farm" the zooxanthellae housed within their siphonal tissues as a holozoic food source. This being strictly so, why are tridacnid amoebocytes culling degenerate rather than young or mature zooxanthellae for subsequent intracellular digestion? From solely the nutritional standpoint, this arrangement doesn't make good sense. While mature zooxanthellae possess storage starch and a nutrient rich cytoplasm, senescent forms are usually comprised of a membranous bag containing little more than calcium oxalate crystals plus the cell wastes (the accumulation bodies) of numerous generations of zooxanthellae (see Freudenthal, 1962; McLaughlin and Zahl, 1966 for details of reproductive biology of zooxanthellae). No doubt there is some nutritional value in a degenerate or senescent zooxanthellae, but surely nothing comparable to that found in a younger cell. Why then do amoebocytes appear to be selectively removing senescent zooxanthellae?

In Taylor's (1969b) study on regulation and maintenance of zooxanthellae in the coelenterate *Anemonia sulcata*, he notes that it is possible for degenerate algal

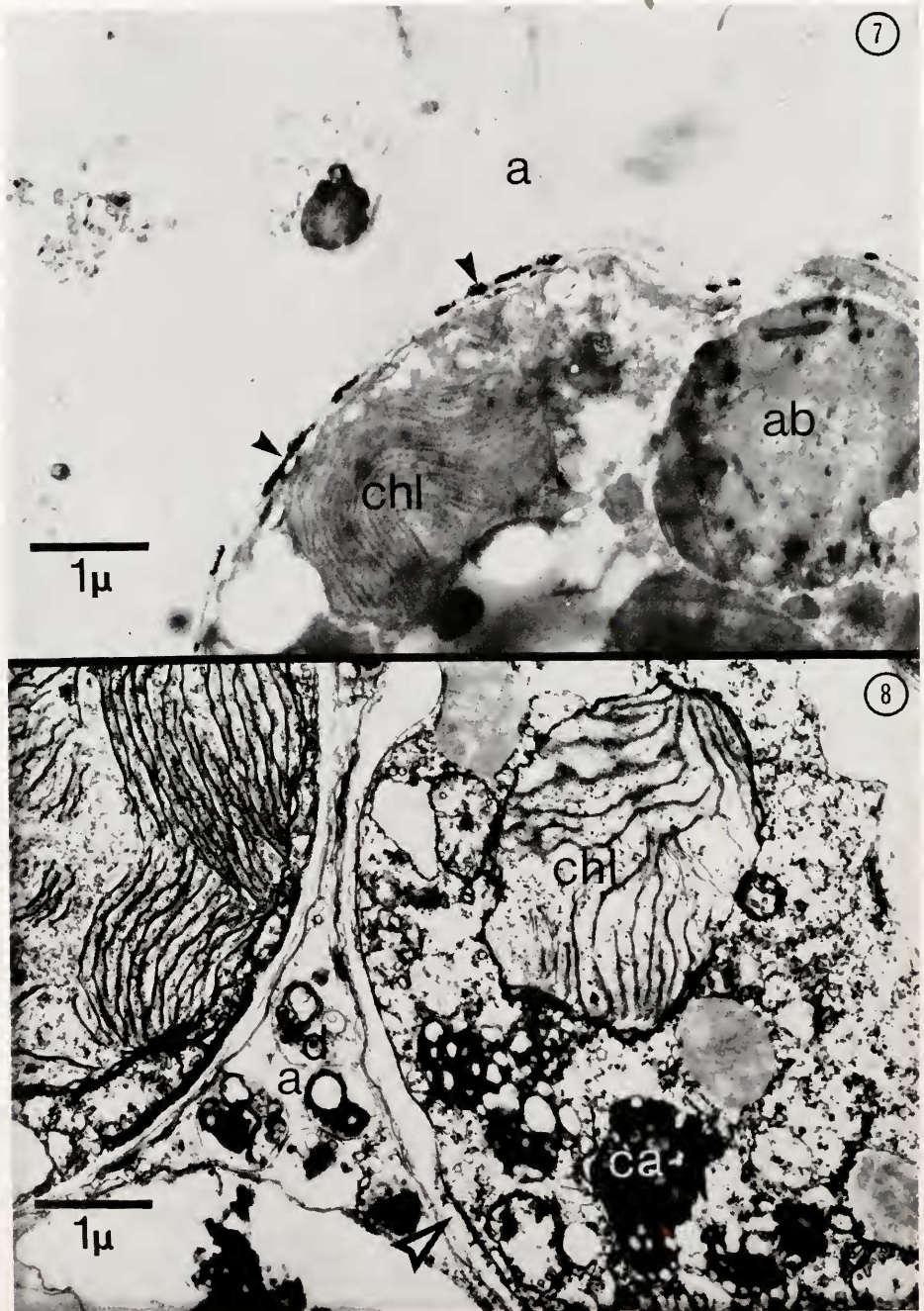


FIGURE 7. Unstained thin section of digestive gland tissue from *Hippopus hippopus* demonstrating sites of phosphomonoesterase II activity (points) during early stage of intracellular digestion of zooxanthella by amoebocyte lysosomes. Abbreviations used are: a, portion of amoebocyte; ab, accumulation body; chl, chloroplast.

cells to differ metabolically from healthy symbionts and, thus, represent a hazard to their host. *Anemonia* rids itself of degenerate zooxanthellae in the same manner as madreporarian corals (Yonge and Nicholls, 1931), by releasing them directly into the environment and thereby maintains a non-harmful host-symbiont balance. Tridacnid clams may confront a similar stress with a different solution—the use of amoebocytes to remove and dispose of zooxanthellae which no longer contribute to the clam's well being.

The criteria for selection or culling of zooxanthellae for sacrifice by tridacnid amoebocytes may well be due to some new expression of the alga's metabolism brought on by senility or degeneration. This new expression of metabolism (*i.e.*, change in a zooxanthella's outer membrane structure and/or a reduction in its release of photosynthetic metabolites to the host) might cause amoebocytes to recognize a senescent zooxanthella as neither host nor symbiont tissue and, thereupon, initiate phagocytosis to remove it from the clam's system.

Yonge (1936) argues in support of "farming" of zooxanthellae by tridacnids through calling attention to the immense size of tridacnid kidneys. He presumes that these organs, which are proportionally a magnitude in size larger than those found in other Lamellibranchs, store the indigestible wastes accumulating from countless instances of amoebocyte digestion of siphonal zooxanthellae. However, growth studies by Rosewater (1965) on *T. gigas* indicate that very large specimens of this clam are at least a quarter of a century old. It appears manifest that by adding this temporal consideration to Yonge's arguments, they become nearly untenable—it is more than a little difficult to visualize how the waste accumulation resultant from 25 years of "farming" could be housed within the kidney spaces of even a very large tridacnid clam.

Results of $^{14}\text{CO}_2$ labeling experiments on *Tridacna maxima* (Goreau, Goreau, and Yonge, 1965) suggest that the turnover rate of zooxanthellae in the interdiverticular spaces of the digestive gland is slow. Coupled with the results of the present paper, this suggests that while there may exist a true holozoic relationship between tridacnid clams and zooxanthellae, this relationship *cannot* be considered "farming," but rather the systematic removal *and utilization* of degenerate zooxanthellae from the mantle's algal population. In this respect, tridacnid clams appear to demonstrate a clear bioenergetic superiority over their coelenterate counterparts.

The evolution of hypertrophied siphons in tridacnids has allowed both greater exposure to solar radiation for proliferation of their algal populations (Yonge, 1936 and 1953) and the development of an extensive microvillous surface, which appears to possess a prodigious capacity for assimilation of both fluid and particulate matter from the surrounding seawater. The ultrastructure of the siphons of *Tridacna crocea* has been described by Kawaguti (1966), who mentions the presence of microvilli covering the siphonal epidermis in passing, but attaches no functional significance to these membranous structures.

Uptake of nutrient material from seawater by microvillous epidermal cells has been clearly established in echinoderms, molluscs, and pogonophores (Fontaine

FIGURE 8. Digestion of zooxanthellae by amoebocyte lying within the interdiverticular spaces of the digestive gland of *Tridacna maxima*. Abbreviations used are: a, portion of amoebocyte; ca, calcium oxalate crystals; chl, chloroplast; d, myelin figures in old digestive vacuole; white on black point, vesicle formation in zooxanthella's periplast.

and Chia, 1968; Little and Gupta, 1968; Pasteels, 1968; Southward and Southward, 1968). Hence, a similar phenomenon in tridacnids is not novel except possibly in a functional sense. For example, absorption of particulate material by the siphonal surface in tridacnids might contribute directly to the nutrition of the siphon's epidermal cells, but does it necessarily follow that the same function is present in the uptake of fluids?

Zooxanthellae have nutrient salt requirements which might be gratified by means of uptake from the adjacent seawater by the epidermal cells of the hypertrophied siphons. Yonge (1936) has found phosphorus metabolism in *Tridacna crocea* strikingly different from that of the tropical bivalve *Spondylus* in that, not only does *T. crocea* remove significant amounts of phosphorus from its environment, but, unlike *Spondylus*, it also retains the phosphorus excretion products of its own protein catabolism. Yonge attributes these metabolic differences to the demanding nutrient salt requirements of the tridacnid's zooxanthellae. This idea of a strong physiological dependence on phosphorus by zooxanthellae gains additional support from the more contemporary findings of McLaughlin and Zahl (1966) who observe that the population structure of axenically cultured zooxanthellae suffers deleterious effects when grown in culture medium which is phosphate depleted.

What, then, might be the pathway of phosphorus removal from seawater? In the case of some non-tridacnid bivalves, the majority of nutrient salts are simply drunk and later absorbed through the gut walls (Allen, 1970; Fretter, 1953). However, in comparison to the greater portion of the Bivalvia, the tridacnid alimentary tract is categorically small in relation to its total biomass. This aspect, in addition to the apparent extra phosphorus requirements of its symbiont algae, suggests that most salt uptake must enter from another site. At present, the microvillous epidermis of the hypertrophied siphons is the only tridacnid tissue in external contact which appears to be clearly capable of fulfilling this critical role. Further, in terms of conservation of metabolic energy, the siphonal epidermis would likely be the most direct path to the zooxanthellae for the transport of nutrient salts. In any event, while I suggest the possibility of the microvillous epidermis being involved in uptake of phosphorus, this is pure speculation which will require the affirmative results of ^{32}P pulse labeling for initial substantiation.

I thank Dr. Philip Helfrich, Director of the Eniwetok Marine Biological Laboratory, for providing logistics and facilities necessary for this work. A portion of the travelling expenses were absorbed by NRC Operating Grant A1427 to Professor G. O. Mackie. Thanks are also due to Sir C. Maurice Yonge for his critical reading of this manuscript.

ADDENDUM (Received August 7, 1971)

I have been recently informed in a personal communication from Sir C. Maurice Yonge of the University of Edinburgh that during the period 1962-63 he and the late Thomas F. Goreau of the University of the West Indies completed a series of experiments with *Tridacna elongata* (= *Tridacna maxima*) utilizing radioactively labeled compounds. The results of this study (shortly to be published in their completed form) revealed that both ^{14}C (presumably in the form of bicarbo-

nate) and tritiated leucine were absorbed through the siphonal surface of *T. elongata*, the latter in great quantities. These data clearly substantiate my interpretation of the ultrastructural features of the tridacnid siphons and that, indeed, material is pinocytosed in prodigious amounts through the microvillous border of the siphonal epidermis. Moreover, Johannes (1967), whose study site was within several yards of where I collected the tridacnids used in this paper, has observed that coral reef waters transport organic nutrient material across the leeward inner reef edge accounting for nearly 2% of the total reef community productivity. Hence, it is apparent that adequate nutrient material is available *in situ* which could be absorbed through the tridacnid siphonal epidermis. These observations possibly weaken Yonge's (1953) thesis that the hypertrophied condition of tridacnid siphons evolved *directly* from this bivalve's association with zooxanthellae.

SUMMARY

The question of utilization of zooxanthellae as a holozoic food source by their tridacnid clam hosts was explored utilizing techniques of electron microscopy and electron microscopical histochemistry. It is apparent from the results that older or senescent zooxanthellae are selectively culled from the algal population of the mantle edge by amoebocytes and are intracellularly digested via amoebocyte lysosomes both in the circulatory system and the interdiverticular spaces of the digestive gland. This process cannot be considered "farming," as figured by earlier work, but rather the slow systematic removal and utilization of degenerate zooxanthellae from the algal population of the clam's mantle edge.

Electron photomicrographs of the microvillous surface of the hypertrophied siphons of the *Tridacna* revealed extensive pinocytosis of fluid and particulate material from seawater bathing the clam. It is suggested *a priori* that this endocytosed material contributes to the nutrition of the clam.

LITERATURE CITED

- ALLEN, J. A., 1970. Experiments on the uptake of radioactive phosphorus by bivalves and its subsequent distribution within the body. *Comp. Biochem. Physiol.*, **36**: 131-141.
- BUCHNER, P., 1965. *Endosymbiosis of Animals with Plant Microorganisms*. [Revised English version] Interscience Publishers, New York, 909 pp.
- DE DUVE, C., 1963. The lysosome. *Sci. Amer.*, **208**: 64-72.
- DOREY, A. E., 1965. The organization and replacement of the epidermis in acoelous turbellarians. *Quart. J. Microscop. Sci.*, **106**(2): 147-172.
- FONTAINE, A. R., AND F. CHIA, 1968. Echinoderms: An autoradiographic study of assimilation of dissolved organic molecules. *Science*, **161**: 1153-1155.
- FRETTER, V., 1953. Experiments with radioactive strontium (^{90}Sr) on certain molluscs and polychaetes. *J. Mar. Biol. Ass. U. K.*, **32**: 367-384.
- FREUDENTHAL, H. D., 1962. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: taxonomy, life cycle, and morphology. *J. Protozool.*, **9**(1): 45-52.
- GOMORI, G., 1950. An improved histochemical technic for acid phosphatase. *Stain Tech.*, **25**(2): 81-85.
- GOREAU, T. F., N. I. GOREAU AND C. M. YONGE, 1965. Evidence for a soluble algal factor produced by the zooxanthellae of *Tridacna elongata* (Bivalvia, Tridacnidae). Unpublished abstract of paper presented to the *International Conference on Tropical Oceanography*, Miami, 1965. [Available from Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh, Scotland.]

- JOHANNES, R. W., 1967. Ecology of organic aggregates in the vicinity of a coral reef. *Limnol. Oceanog.*, **12**: 189-195.
- KAWAGUTI, S., 1966. Electron microscopy on the mantle of the giant clam with special references to zooxanthellae and iridophores. *Biol. J. Okayama Univ.*, **12**(3-4): 81-92.
- KEVIN, M. J., W. T. HALL, J. J. A. McLAUGHLIN AND PAUL A. ZAHL, 1969. *Symbiodinium microadriaticum* Freudenthal, a revised taxonomic description, ultrastructure. *J. Phycol.*, **5**(4): 341-350.
- LITTLE, C., AND B. L. GUPTA, 1968. Pogonophora: uptake of dissolved nutrients. *Nature*, **218**: 873-874.
- LUFT, J., 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.*, **9**: 409-414.
- MANSOUR, K., 1945. The zooxanthellae, morphological peculiarities and food and feeding habits of the Tridacnidae with reference to other lamellibranchs. *Proc. Egypt. Acad. Sci.*, **1**: 1-11.
- MANSOUR, K., 1946. Source and fate of the zooxanthellae of the visceral mass of *Tridacna elongata*. *Nature*, **158**: 130.
- MANSOUR, K., 1949. The morphological and biological peculiarities of *Tridacna elongata* and *T. squamosa*. *C. R. 13th Congr., Int. Zool. Paris*, **1948**: 441-444.
- McLAUGHLIN, J. A., AND P. A. ZAHL, 1966. Endozoic Algae. Pages 257-297 in S. Mark Henry, Ed., *Symbiosis, Volume 1*. Academic Press, New York.
- PASTEELS, J. J., 1968. Pinocytose et athrocytose par l'épithélium branchial de *Mytilus edulis* L. *Z. Zellforsch.*, **92**: 339-359.
- RICHARDSON, K. C., L. JARETT AND E. H. FINKE, 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Tech.*, **35**(6): 313-323.
- ROSEWATER, J., 1965. The family Tridacnidae in the Indo-Pacific. *Indo-Pacific Mollusca*, **1**(6): 347-396.
- SMITH, D., L. MUSCATINE AND D. LEWIS, 1969. Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. *Biol. Rev.*, **44**: 17-90.
- SOUTHWARD, A. J., AND E. C. SOUTHWARD, 1968. Uptake and incorporation of labelled glycine by pogonophores. *Nature*, **218**: 875-876.
- TAYLOR, D. L., 1968. *In situ* studies on the cytochemistry and ultra-structure of a symbiotic marine dinoflagellate. *J. Mar. Biol. Ass. U. K.*, **48**: 349-366.
- TAYLOR, D. L., 1969a. Identity of zooxanthellae isolated from some Pacific Tridacnidae. *J. Phycol.*, **5**(4): 336-340.
- TAYLOR, D. L., 1969b. On the regulation and maintenance of algal numbers in zooxanthellae-coelenterate symbiosis, with a note on the nutritional relationship in *Ancmonia sulcata*. *J. Mar. Biol. Ass. U. K.*, **49**: 1057-1065.
- YONGE, C. M., 1936. Mode of life, feeding, digestion and symbiosis with zooxanthellae in the Tridacnidae. *Sci. Rept. Great Barrier Reef Exped.*, **1**(11): 283-321.
- YONGE, C. M., 1944. Experimental analysis of the association between invertebrates and unicellular algae. *Biol. Rev.*, **19**: 68-80.
- YONGE, C. M., 1953. Mantle chambers and water circulation in the Tridacnidae (Mollusca). *Proc. Zool. Soc. London*, **123**: 551-561.
- YONGE, C. M., AND A. G. NICHOLLS, 1931. Studies on the physiology of corals. V. The effect of starvation in light and in darkness on the relationship between corals and zooxanthellae. *Sci. Rept. Great Barrier Reef Exped.*, **1**(7): 177-211.